REMARKS

Claims 58-79 are currently pending in the application. Claims 59, 61-65, 67, 69-72, 77-79 are canceled. Claims 58, 60, 66, and 73-76 are amended. The amendments find support in the specification and are discussed in the relevant sections below. No new matter is added.

Applicants thank the Examiner for graciously responding to Applicant's questions regarding the outstanding response. Applicants appreciate the Examiner's willingness to search a combination of three genes of the original elected combination of ten genes, despite the Examiner's having already searched the ten sequences as a combination.

Claim Amendments

Support for methods of diagnosing osteoarthritis, mild osteoarthritis and severe osteoarthritis comprising the elected combination of three genes, Tumor Necrosis Factor Alpha-induced Protein (TNFAIP6); Calmodulin 1 (CALM1); and Laminin, gamma 1 (LAMC1), is found throughout the specification, in particular in Figure 6.

Specifically support for the phrase "wherein the step of determining said levels of said RNA transcripts in said cartilage sample from said individual suspected of having or being afflicted with OA, comprises hybridizing a nucleic acid sample from said cartilage sample" is found throughout the specification, specifically on page 10, first paragraph.

Support for the phrase "level of expression of RNA transcripts of a gene" is found throughout the specification, including: page 21, paragraph 285-286.

Support for the phrase "gene expression pattern" is found through the specification including: page 9 paragraph 139, and page 20 paragraph 275, and page 29.

Support for the method comprising the step of determining said levels of said RNA transcripts in said cartilage sample from said individual suspected of having or being afflicted with OA, comprising hybridizing a nucleic acid sample from said cartilage sample, is found throughout the specification, including on page 10, first paragraph.

Applicants respectfully request that the amended drawings, replacement CD and identical copy thereof, all of which were filed and entered with Applicant's response in December 2005, be considered with the instant filing. Applicants respectfully request entry of a new §1.132 declaration by Honwei Zhang which accompanies this reply.

Claim Objections

Claims 58-73 are objected to because they refer to the Figures, and in particular Figure 6. As stated, incorporation by reference of a table is permitted in the exceptional circumstances where there is no practical way to define the invention in words, and where it is more concise to incorporate by reference rather than duplicating a drawing or table into the claim.

Applicant has canceled claims 59, 61-65, 67, and 69-72, and amended the remaining instant claims so that they do not refer to the Figures, and so they recite the names of the 3 elected genes, Tumor Necrosis Factor Alpha-induced Protein (TNFAIP6), Calmodulin 1 (CALM1), and Laminin, gamma 1 (LAMC1).

In view of this amendment, Applicant respectfully requests reconsideration and withdrawal of the objection.

Specification

It is suggested that Figures which contain text concerning the differential expression of genes in OA be converted into Tables so as to permit the information to be search accessible by the public. The Applicant is taking this recommendation under consideration.

Drawings

As noted in the Office action, Figures 14 and 14A were removed by Applicant, the information therein being transferred to a sequence listing. In view of the removal of Figures 14 and 14A, the pages of the drawings have been renumbered, and the specification has been amended to delete references to Figures 14 and 14a. The specification has also been amended to

reflect the renumbered figures including amendment of the brief description of the drawings section.

Compact Disc Submission

A replacement CD and identical copy thereof, with the sequence listing and sequence statemments, was submitted with the response filed December 2005. No new matter has been added. This sequence listing is identical to now cancelled Figure 14.

35 USC 112 Rejections

Indefiniteness

Claims 58-73 are rejected under 35 USC 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

The Examiner objects to the language "RNA transcripts which correspond to a gene" recited in claims 58-73 as being indefinite on the basis that it is not clear what RNA transcripts are meant to be encompassed by the claims.

Applicant has canceled claims 59, 61-65, 67, and 69-72 and amended the remaining instant claims so that they do not recite the language "RNA transcripts which correspond to a gene".

The Examiner further states that the claims are indefinite "because it is not clear what is meant by the genes 'selected from Figure 6' ". Applicant has canceled claims 59, 61-65, 67, and 69-72 and amended the remaining instant claims so that they do not recite that the genes are "selected from Figure 6".

In view of the claim amendments and Applicant's arguments, reconsideration and withdrawal of the rejection is respectfully requested.

Enablement

Claims 58-73 are rejected under 35 USC 112, first paragraph, as failing to comply with the enablement requirement.

Examiner objects to claims 58-73 as failing to comply with the enablement requirement and in particular cites (a) the Nature of the Invention (b) the Scope of the Invention (c) the Guidance in the Specification and (d) the Teachings in the Prior Art and Level of Unpredictability and (e) Quantity of Experimentation. Applicant will address each of the points raised below.

Nature of the Invention

Examiner states that the claims recite a method of diagnosing osteoarthritis or a stage of OA which, as a result of the Examiner's Restriction Requirement, requires determining the level of the ten genes selected. Applicant has canceled claims 59, 61-65, 67, and 69-72, and has amended the remaining instant claims so they recite a method requiring a determination of the level of RNA of the three genes selected; Tumor Necrosis Factor Alpha-induced Protein (TNFAIP6), Calmodulin 1 (CALM1), and Laminin, gamma 1 (LAMC1). Reconsideration of this issue is respectfully requested.

The Examiner further indicates that the nature of the claimed invention requires knowledge that the genes are differentially expressed in OA or a stage of OA in such a way that one can reliably draw conclusions for the diagnosis of OA based on the gene expression patterns.

It is the Applicant's position that, the use of EST frequency to draw conclusions regarding differential expression is a scientifically acceptable technique (see for example Okubo *et al. Nature Genetics* **2**, 173 - 179 (1992), Kumar S, Connor JR, Dodds RA, Halsey W, Van Horn M, Mao J. et al. Osteoarthritis Cartilage. 2001 Oct;9(7):641-53; Dahl et al. The Journal of Pathology 2005 205 (1) 21-28). Further evidence in support of this position is provided by way of an Inventor's Declaration wherein additional data is provided. In summary, the data demonstrates that additional screening of the described cDNA libraries continues to support the

biomarkers of Figure 6, in particular the three biomarkers Tumor Necrosis Factor Alpha-induced Protein (TNFAIP6), Calmodulin 1 (CALM1), and Laminin, gamma 1 (LAMC1), as being differentially expressed as between OA and non OA samples.

In addition, using a second technique of either Affymetrix® microarray and/or ChondroChip™ microarray hybridization resulted in data which demonstrates the biomarkers of Figure 6, in particular the biomarkers Tumor Necrosis Factor Alpha-induced Protein (TNFAIP6), Calmodulin 1 (CALM1), and Laminin, gamma 1 (LAMC1), are differentially expressed as between OA and non OA. The analysis of the data is discussed more thoroughly in the section entitled "Guidance in the Specification and Working Examples".

Scope of the Invention

The Examiner indicates that the scope of the language used in the claims is sufficiently broad to encompass (a) any sample including blood, synovial fluid and cartilage, (b) all homologues, variants and the like, and (c) all species of patient. In order to expedite prosecution, but without prejudice to the Applicant's rights to pursue related claims, Applicant has amended the claims so as to limit them to the diagnosis of human osteoarthritis using cartilage samples. Applicant has clarified that the transcripts which are expressed from the recited three biomarker genes are included within the scope of the claim. This would therefore include all transcripts which are expressed in cartilage from the recited three biomarker genes, Tumor Necrosis Factor Alpha-induced Protein (TNFAIP6), Calmodulin 1 (CALM1), and Laminin, gamma 1 (LAMC1). This is consistent with the teaching within the specification wherein Applicant has identified numerous transcripts, or portions thereof, transcribed from the genes as shown in Figure 6.

Guidance in the Specification and Working Examples

Examiner argues that before reliable conclusions can be drawn regarding diagnosis or staging of OA, there are a number of issues that need to be addressed including those that follow: (a) reliability of the EST frequency data to demonstrate differential gene expression (b) the lack of working examples of the claimed method for use in diagnosing of OA.

With regards to the former, as already mentioned, EST frequency data to draw conclusions regarding differential expression is a scientifically acceptable technique. The EST frequency analysis was done by analyzing and sequencing over 50,000 EST transcripts in a normal cartilage library, a mild OA cartilage library, and a severe OA cartilage library. Each of these ESTs was sequenced and matched to known genes where possible. As outlined in the Inventor's Declaration each of the cDNA libraries was constructed from two or more individuals and in particular the normal OA cartilage library was constructed using mRNA isolated from two individuals, the mild OA cartilage library was constructed using RNA isolated from six individuals, the severe OA cartilage library was constructed using RNA isolated from 3 individuals.

The decision in *In re Angstadt*, 190 U.S.P.Q. 218 (C.C.P.A. 1976) clearly states that every embodiment need not be disclosed, even in an unpredictable art, and clearly permits the presence of a screening step to identify those embodiments which possess the desired activity. In fact, in *Angstadt*, the Court specifically dismissed the notion that the specification must provide a level of guidance that would predict the outcome of an experiment (or reaction) "with reasonable certainty before performing the reaction" and that "such a proposition is contrary to the basic policy of the Patent Act, which is to encourage disclosure of inventions and thereby to promote progress in the useful arts."

Since filing of the patent application, Applicant has continued to identify ESTs from the four cDNA libraries following the methodologies as outlined in the specification. As can be seen in the Inventor's Declaration, in most cases where additional ESTs were identified, the EST frequency as between osteoarthritis libraries and normal libraries show the same trend (i.e. upregulated and or downregulated when comparing osteoarthritis to non osteoarthritis) as the original EST frequency data. This is true even in those cases where, as the Examiner notes, the number of ESTs identified originally is relatively few (see for example LAMC1; and IL13RA1).

The Applicant also provides additional data obtained using microarray analysis of the elected genes Tumor Necrosis Factor Alpha-induced Protein (TNFAIP6), Calmodulin 1 (CALM1), and Laminin, gamma 1 (LAMC1), to further support their utility as biomarkers.

Microarray analysis was performed using the methods as taught in the specification and hybridizing to either the Affymetrix GeneChip® and/or the Applicant's own ChondroChip® constructed from some of the EST's identified. In particular RNA was isolated from the cartilage of numerous individuals having osteoarthritis and numerous individuals not having osteoarthritis, and each RNA sample converted into cDNA for purposes of hybridization to the arrays. For most of the elected genes, the hybridization data is consistent with the EST frequency analysis and demonstrates statistical significant (i.e. a p value of less than 0.05) in differential expression as between individuals having osteoarthritis (where at least 7-10 individuals with osteoarthritis were used for the analysis of any one selected biomarker) and individuals not having osteoarthritis (where at least 10 individuals with osteoarthritis were used for the analysis of any one selected biomarker).

In three instances (B2M and ZFR, and TCTP) the data obtained by hybridization does not concord with the EST frequency data. As is understood, the law clearly does not require all of the species embodied within the scope of a claim to be operative for a claim to be valid (Atlas Powder Co. v. E.I. du Pont de Nemours & Co., 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984)). The microarray results still, however, in the case of B2M and ZFR demonstrate that the products of these genes are differentially expressed as between individuals having osteoarthritis and not having osteoarthritis.

Examiner further claims there is no guidance in the specification as to how the genes of the invention can be used for the diagnosis of OA. Applicant respectfully disagrees. The specification teaches the use of the genes of the invention for diagnosis of OA starting on page 21 paragraph 289-292. The specification further provides Example 9 as a working example of the invention in diagnosing OA. More specifically, the specification teaches isolation of an RNA sample from a test individual and hybridization of the RNA to a microarray comprised of nucleic acid members wherein at least one of the members corresponds to a gene which is identified as differentially expressed in individuals having osteoarthritis as compared to "normal" individuals (i.e. individuals not having osteoarthritis) to generate a gene expression pattern. As described in the definition of "indicative of disease" found on page 9, paragraph 133, an expression pattern is

diagnostic if it is found significantly more often in patients with the disease than in patients without the disease using standards routine statistical methods. Thus the specification teaches how to use the genes of the invention for diagnosis of OA. The reconsideration and withdrawal of the rejection is respectfully requested.

Teachings in the Prior Art and Level of Unpredictability

Examiner further argues that is highly unpredictable whether the differential expression observed is due to OA or a stage of OA or if it represents a more generalized response to other conditions. With respect, Applicant disagrees. The genes of the invention were identified as those which are differentially expressed in the tissue at the site of the osteoarthritic decay, where it is known that the structural integrity of mature cartilage is in a delicate balance. Thus, it is expected that changes in the expression of the genes at this cartilage site are relevant to osteoarthritis and useful in its diagnosis. Furthermore, we identified many of the genes as being differentially expressed not only in the cartilage of osteoarthritic patients as compared with non osteoarthritic patients, but also as between different stages of osteoarthritis – again showing the likelihood that the differential expression of these genes is due to osteoarthritis.

As stated in the Manual of Patent Examining Procedure at 2164.03:

The "predictability or lack thereof" in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. If one skilled in the art can readily anticipate the effect of a change (in this case a change in gene expression in the cartilage of osteoarthritic patients as compared with non osteoarthritic patients) within the subject matter to which the claimed invention pertains, then there is predictability in the art.

As evidenced by Exhibit "A" in the attached declaration, there is a high degree of correlation between the elected three biomarker genes and their ability to monitor differential expression as between the cartilage of osteoarthritic patients as compared with the cartilage of non osteoarthritic patients. Absent evidence to the contrary, there is ample support for the conclusion that one skilled in the art would be able to extrapolate the results of the claimed invention including; (a)differential expression occurs in cartilage, (b) many of the genes

identified are also differentially expressed as between different stages of osteoarthritis, (c) later correlating data obtained following the teachings as disclosed in the specification confirming the conclusion that the differential expression is indicative of osteoarthritis. Since the examiner has presented no evidence to the contrary, and one skilled in the art has the ability to anticipate the effect of the differential expression of the claimed genes to the claimed method of diagnosing osteoarthritis, predictability exists in the art.

Quantity of Experimentation

The Examiner suggests that the level of experimentation required to practice this invention are too enormous. In *In re Wands*, the court stated that "[e]nablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. 'The key word is 'undue' not 'experimentation' (citing *In re Angstadt*, 537 F. 2d 498 at 504, 190 U.S.P.Q. 214 at 219 (C.C.P.A. 1976)). The Court also stated that "the test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." (citing *In re Jackson*, 217 U.S.P.Q. 804 at 807 (Bd. App. 1982)).

Applicant has already demonstrated that in their own work, they have been able to perform the experimentation necessary to determine that the genes elected are differentially expressed in normal patients as compared with those patients having osteoarthritis using microarray technology. This powerful technology allows one to test for expression of over 30,000 genes in one experiment for cartilage samples of numerous individuals, therefore Applicant has demonstrated that the experimentation necessary is not undue. In fact, all of the genes shown in Figure 6 can be analyzed in a single experiment for any individual.

Examiner further suggests that one would also have to show that the patterns of differential expression are specific to Osteoarthritis and not due to other diseases. Examiner indicates that in order to support a claim using these genes for diagnosis of OA, one would have

to show that the genes are not differentially expressed in other disease areas. Applicant would point out that diagnosis, as is understood by a person skilled in the art, is not performed in the absence of other medical information including past history, symptoms, and the like. Therefore, it is not necessary that the biomarkers be tested to ensure that, for example, a person with a broken leg does not show the same pattern of differential expression. Diagnosis is often done in combination with many other factors and tests. For example, commercially available tests for Rheumatoid Arthritis are routinely used despite the possibility of these tests indicating other possible conditions.

Reconsideration and withdrawal of the rejection is respectfully requested

Conclusion

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

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